Histopathological and Histochemical Effects of Larval Trematodes in *Goniobasis virginica* (Gastropoda: Pleuroceridae)

by

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Abstract. The histopathological and histochemical effects of parasitism in *Goniobasis virginica* (Gmelin, 1791) (Gastropoda: Pleuroceridae) by the rediae of *Sphaeridiotrema globulus* and *Philophthalmus megalurus* and the microphallid sporocysts of a ubiquita type and a lecithodendriid of a virgulate type are reported. The larval stages caused extensive damage to the digestive gland and the muscle surrounding the gland either by ingesting host tissue or through increased pressure due to their sheer numbers. There was no detectable hemocyte response by the molluscan host to the presence of living rediae and sporocysts. The occurrence of dead rediae of *S. globulus* did elicit a hemocyte response. Rediae of *S. globulus* were found also in kidney and gill tissue. Decreases in glycogen, lipid, acid mucopolysaccharide, and keratin content of the digestive gland occurred in parasitized snails. Amyloid content was unchanged in parasitized snails. Double infections involving *S. globulus* and microphallid sporocysts, and *P. megalurus* and microphallid sporocysts, also occurred in *G. virginica*. Metacercariae of *S. globulus* are found free between the shell and visceral mass and do not elicit any discernable pathologic response in the snail.

INTRODUCTION

VARIOUS HISTOPATHOLOGICAL and histochemical investigations have been made on digenean larvae and their molluscan hosts (JAMES, 1965; WRIGHT, 1966; READER, 1971a, b). Previous studies have dealt with a variety of mollusks (HURST, 1927; PRATT & BARTON, 1941; CHENG, 1963a, b; PORTER et al., 1967; MOORE & HALTON, 1973; BECKER, 1980) but none have involved the pleurocerid gastropod *Goniobasis virginica* (Gmelin, 1791).

Goniobasis virginica is the intermediate host for *Sphaeridiotrema globulus* in Lake Musconetcong, New Jersey (HUFFMAN & FRIED, 1983). Adults of this fluke produce ulcerative hemorrhagic enteritis in mute swans and have accounted for 142 deaths of these birds at Lake Musconetcong between September 1977 and December 1981 (ROSCOE & HUFFMAN, 1982). During the survey of *G. virginica* by HUFFMAN & FRIED (1983), four species of larval trematodes were found in this snail.

In the present investigation, a comparative study has been made on the pathological and histochemical effects of the rediae of *Sphaeridiotrema globulus* and *Philophthalmus megalurus,* the sporocysts of a microphallid of a ubiquita type, and a lecithodendriid of a virgulate type (two different types of microphallids) on the digestive gland, muscle, kidney, and gill of *Goniobasis virginica.* Stained sections of uninfected snail tissues were compared with similarly stained sections of parasitized tissue.

The morphology and function of gastropod digestive gland cells have been disputed. SUMNER (1965) described four morphologically distinct types of cells. BARFURTH (1880), READER (1971a), and MOORE & HALTON (1973)

recognized only three, while PORTER *et al.* (1967) and PORTER (1970) reported two distinct cell types in *Oxytrema siliqua* and *Flumenicola virens* respectively. Examination of existing reports also revealed that the same cell type is designated by an assortment of names.

The studies reported here were initiated to determine (1) histologically the loci of different trematode infections within the snail host; (2) if the pathological and histochemical response varies depending upon the species of larval trematode involved; and (3) the morphology of normal digestive gland of *Goniobasis virginica* as a base for evaluating pathologic responses to larval trematode infections.

MATERIALS AND METHODS

Specimens of *Goniobasis virginica* ranging in length from 20 to 30 mm were collected from Lake Musconetcong, New Jersey, and maintained in the laboratory in a 38-L filtered aquarium containing lake water. Snails were crushed within 3 days after collection and were examined under a dissection microscope. Forty infected snails were divided into four groups of ten, with each group representing one of the four species of larval trematodes. Two groups of five snails, each doubly infected with *Sphaeridiotrema globulus* or *Philophthalmus megalurus* and a microphallid, were processed for study along with ten uninfected snails. Parasitized and nonparasitized snails were removed from their shells and fixed in 10% neutral buffered formalin (NBF). For histopathological studies, tissues were dehydrated in an alcohol series, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. For the detection of neutral lipids, tissues were fixed in NBF, embedded at −20°C in an inert embedding compound used for cryostat microtomy (O.C.T.; Ames Co., Elkhart, Indiana), and sectioned at 8 μm on a CTF microtome-cryostat (International). For histochemical studies, the tissues were dehydrated in an alcohol series, embedded in paraffin, sectioned at 6 μm, and the following procedures (LUNA, 1968) were used: alcian blue, pH 2.5, 1.0, and 0.4 for acid mucopolysaccharides; periodic acid-Schiff (PAS) reaction for complex carbohydrates, with controls incubated in 0.5% malt diatase; Ayoub-Shklar's method for keratin (sulfur containing fibrous protein); Bennhold's method for amyloid (carbohydrate-containing protein); Oil Red O in propylene glycol for neutral lipids; Dahl's alizarin red S method for calcium salts; Oil Red O method for lipofuchsin; and Gomori's one step trichrome for connective tissue.

RESULTS

Morphology

The uninfected digestive gland of *Goniobasis virginica* is orange or brown and occupies the upper whorls of the shell. The digestive gland consists of numerous tubules surrounded by loose connective tissue containing the visceral hemocoelic space. The tubules are separated from the hemocoel by a thin layer of loose connective tissue and lined with glandular epithelium (Figure 1). This epithelium is composed of two cell types, serous and mucous cells. The serous cells are triangular. The cytoplasm at the base of each cell is basophilic. The nucleus is round and situated close to the base of the cell. The cytoplasm toward the apex of the serous cells contains eosinophilic granules. The mucous cells are columnar with flattened nuclei which are crowded against the base of the cells. There is less basophilia at the base of these cells than in serous cells. The cytoplasm of mucous cells contains glycogen, lipid, and amyloid. The serous cells contain keratin, lipofuchsin, and calcium salts. The periphery of the digestive gland consists of muscle (Figure 2). A membrane, comprised of a single squamous epithelial layer overlying a connective tissue layer, encloses the digestive gland.

Gross Pathology Due to *Sphaeridiotrema globulus*

The digestive glands of snails infected with the rediae of *S. globulus* are white. The rediae are readily visible as robust white organisms, each with an orange longitudinal streak. The streak is the pharynx and gut containing cellular debris of host origin.

Histopathology Due to *Sphaeridiotrema globulus*

The rediae of *S. globulus* are located mainly in the periphery of the digestive gland (Figure 3) and cause extensive damage to the muscle. Rediae are surrounded by clear zones (Figure 4) devoid of cells. There is no detectable hemocytic response on the part of the molluscan host to the presence of living rediae. In snails with dead rediae of *S. globulus,* there is a hemocytic response to the parasite (Figure 5). Dead rediae differ from live rediae in that the parasite's muscular tissue and tegument undergo autolysis with loss of cellular structure. The germinal cells within rediae appear to be more resistant to autolysis and consequently retain their normal appearance. In heavy, and also in double infections involving microphallid sporocysts, rediae are found abutting tubules in the digestive gland, in the kidney, and in gill tissue (Figures 6, 7). Digestive gland tubules in contact with rediae show reduced lumen size. The decrease in size is due to the pressure exerted by large numbers of parasites. Rediae occurring in the digestive gland disrupt the connective tissue network.

The encysted metacercariae of *Sphaeridiotrema globulus* also occur in *Goniobasis virginica* but are free between the shell and visceral mass and do not elicit a discernable pathologic response.

Histochemistry

The digestive gland of uninfected *Goniobasis virginica* contains glycogen. Clumps of PAS-positive granules are
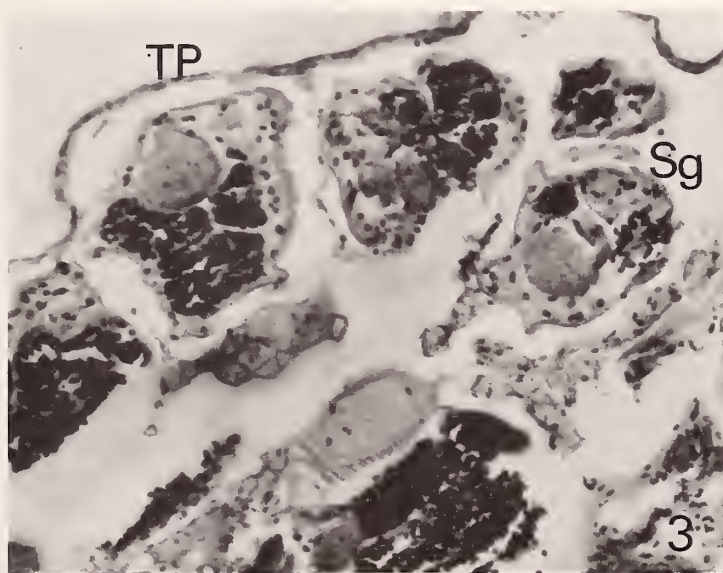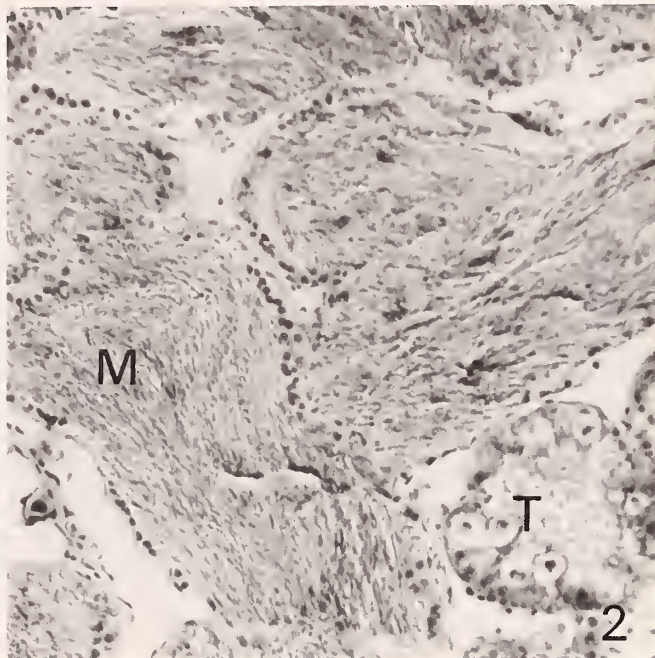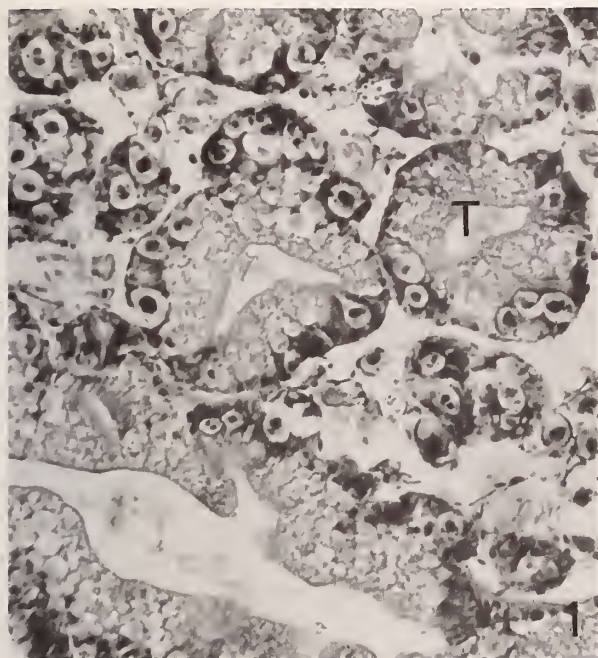
Explanation of Figures 1 to 3

Figure 1. Uninfected *Goniobasis virginica* digestive gland, illustrating tubule (T). H&E, 100×.

Figure 2. Uninfected *Goniobasis virginica* illustrating muscle (M) and tubule (T) of digestive gland. H&E, 100×.

Figure 3. *Sphaeridiotrema globulus* (Sg) rediae in the periphery of the digestive gland of *Goniobasis virginica*, also illustrating tunica propria (TP). H&E, 100×.

present throughout the cytoplasm of the mucous cells of the digestive gland. With the infection of the digestive gland with *Sphaeridiotrema globulus*, glycogen decreases concurrent with the presence of PAS-positive material in the rediae and cercariae of *S. globulus*.

Digestive gland cells in uninfected snails contain keratin. Keratin is present in the rediae and cercariae of *S. globulus* but host stores are not depleted. Dead *S. globulus* rediae are surrounded by a thin layer of keratin.

Acid mucopolysaccharides (pH 2.5, 1.0, 0.4) were pres-
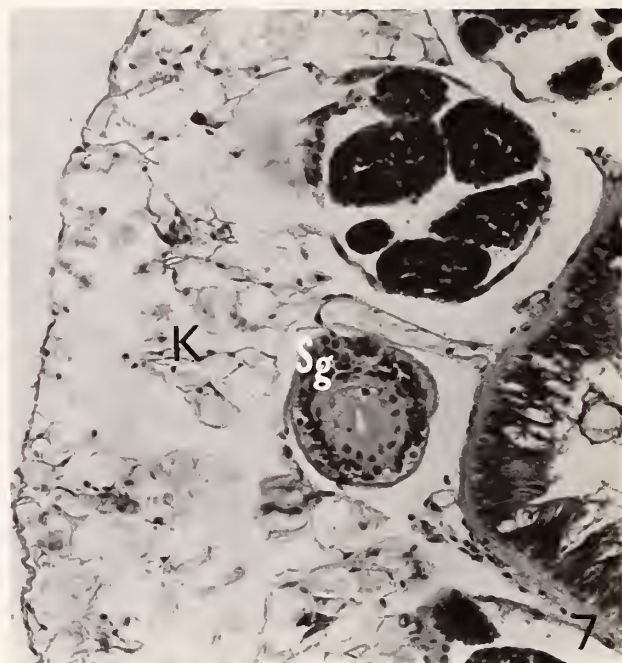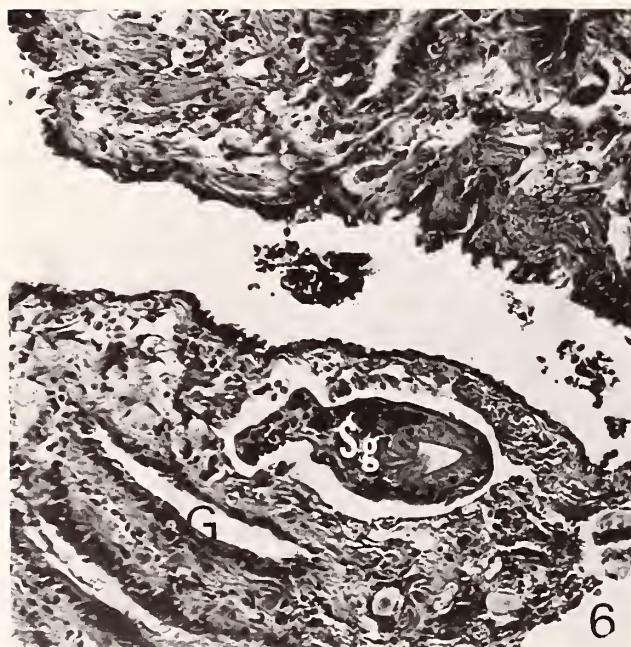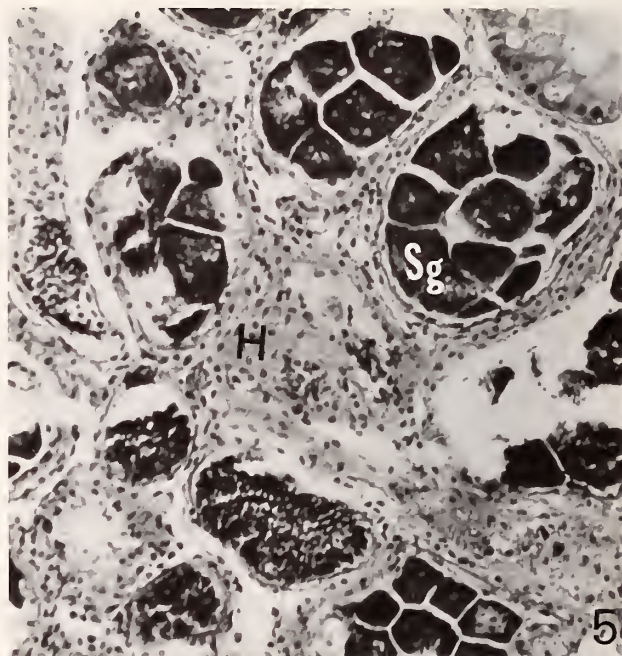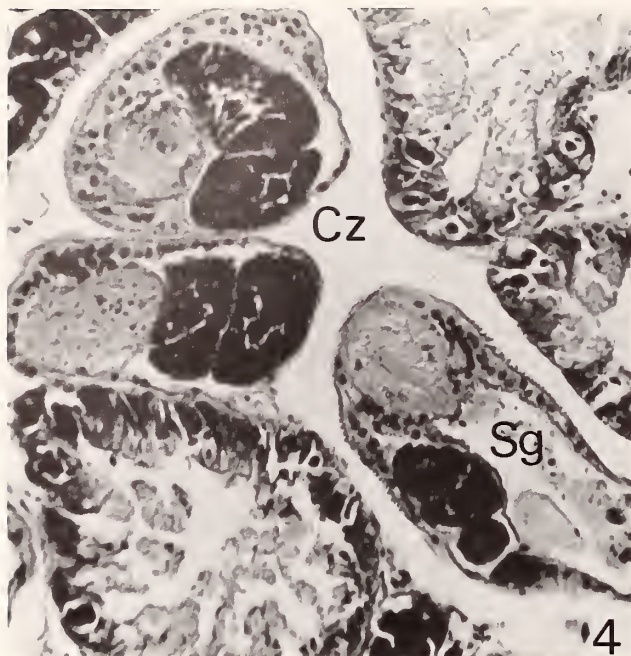
Explanation of Figures 4 to 7

Figure 4. *Sphaeridiotrema globulus* (Sg) in the periphery of the digestive gland illustrating clear zones (Cz) surrounding the parasite. H&E, 100×.

Figure 5. Hemocyte response (H) to dead *Sphaeridiotrema globulus* (Sg). H&E, 450×.

Figure 6. *Sphaeridiotrema globulus* (Sg) redia in gill tissue (G) of *Goniobasis virginica*. H&E, 100×.

Figure 7. *Sphaeridiotrema globulus* (Sg) rediae in kidney (K) of *Goniobasis virginica*. H&E, 100×.

ent in uninfected snail digestive gland tissue and depletion occurs in snails infected with *S. globulus* rediae. Amyloid content is unchanged in parasitized snails.

In uninfected snails, neutral lipids occur in the mucous cells of the digestive gland and scattered throughout intertubular spaces. The neutral lipid content in tubules in close proximity to *S. globulus* rediae is diminished. Lipid occurs in the body wall and gut of these rediae.

Gross Pathology Due to *Philophthalmus megalurus*

The digestive gland of snails infected with the redial stages of *P. megalurus* is white and orange. The rediae are visible to the naked eye. The rediae of *P. megalurus* are more elongate and not as robust as those of *Sphaeridiotrema globulus*.

Histopathology Due to *Philophthalmus megalurus*

The rediae of *P. megalurus* are located in the periphery of the digestive gland and cause extensive disruption of the myofibers in the periphery of the gland (Figure 8). This parasite also invades the digestive gland (Figure 9), causing decreased tubular lumen size due to the pressure exerted by the parasite and disrupting the connective tissue network. There is no host hemocytic response to the living rediae. Rediae are surrounded by clear zones devoid of cells.

Histochemistry

Glycogen and neutral lipid depletion occur in snails infected with *Philophthalmus megalurus*. Glycogen is present within the redial gut and body wall. Only tubules in close proximity to parasites revealed a decrease in neutral lipid content. Neutral lipid occurs in the body wall and gut of the rediae.

Keratin occurs within the rediae and cercariae of *P. megalurus*. The host digestive gland cells and muscles are devoid of the substance. Rediae and cercariae accumulate acid mucopolysaccharides concurrent with depletion from host tissue. Amyloid content is unchanged in parasitized snails.

Gross Pathology Due to Ubiquita Microphallid and Virgulate Lecithodendriid

The daughter sporocysts of the microphallid and the lecithodendriid are not visible to the naked eye; therefore, gross pathology is not discernable. Once the snail tissue is dissected, the small white rounded structures can be seen with a dissecting microscope.

Histopathology Due to Ubiquita Microphallid and Virgulate Lecithodendriid

The microphallid and lecithodendriid sporocysts are located primarily in the visceral hemocoelic spaces of the digestive gland (Figure 10). Digestive gland tubules in contact with sporocysts show a reduced lumen. There is disruption of the connective tissue network. In heavily infected snails, the digestive gland is reduced substantially from its normal size. Sporocysts become intimately associated with the digestive gland tissue and some sporocysts abut the digestive tubules (Figure 12). The thin connective sheath surrounding the tubule remains intact.

Histochemistry

Depletion of acid mucopolysaccharides (pH 2.5, 1.0, 0.4) occurs with both of the sporocyst infections. Abundant amounts were found within the sporocysts. Glycogen and neutral lipid depletion also occurred in both sporocyst infections. Glycogen granules were associated with the sporocyst body wall. Neutral lipids were demonstrated in developing cercariae. Amyloid content was unchanged; trace amounts do appear in the sporocysts.

In double infections (Figure 11), the observations were the same as for single infections involving *Sphaeridiotrema globulus, Philophthalmus megalurus,* and the microphallid. The double infections occupy the periphery of the digestive gland, kidney, and gills of infected snails.

DISCUSSION

Despite numerous studies of the gastropod digestive gland, there is little agreement on the terminology and classification of cell types. PAN (1958) described three cell types in the digestive gland of *Biomphalaria glabrata*: goblet, lime, and digestive. SUMNER (1965) described four types of epithelial cells in *Helix aspersa*. PORTER *et al.* (1967) recognized two types in *Oxytrema siliqua*: liver and calcium cells. READER (1971a) recognized three types in *Bithynia tentaculata*: absorptive, secretory, and thin cells. MOORE & HALTON (1973) described three cell types in *Lymnaea truncatula*: digestive, mucous, and basophil cells.

Absorptive cells have previously been called liver, ferment, digestive, secretory, or excretory cells. Secretory cells have previously been referred to as lime, calcium, or excretory cells. Mucous and serous secreting cell types are present in *Goniobasis virginica*. The epithelial cells of the digestive gland in this gastropod have morphological characteristics typical of mucous and serous secreting columnar epithelium. These two morphologically distinct types are further differentiated on the basis of their cytoplasmic constituents identified by histochemical analysis.

Numerous studies have been made on the destruction of molluscan digestive gland by larval trematodes (FAUST, 1920; AGERSBORG, 1924; HURST, 1927; PRATT & BARTON, 1941; CHENG & JAMES, 1960; CHENG & SYNDER, 1962a, b, 1963; PORTER *et al.,* 1967; READER, 1971b; MEULEMAN, 1972; YOSHINO, 1976; TRIPP & TURNER, 1978). In the present study, the rediae of *Sphaeridiotrema globulus* and *Philophthalmus megalurus,* sporocysts of a microphallid and a lecithodendriid trematode have some pathological effects on the tissues of *Goniobasis virginica*.
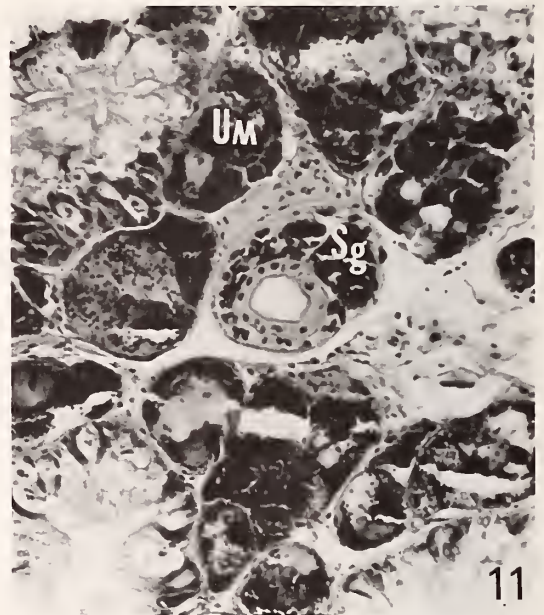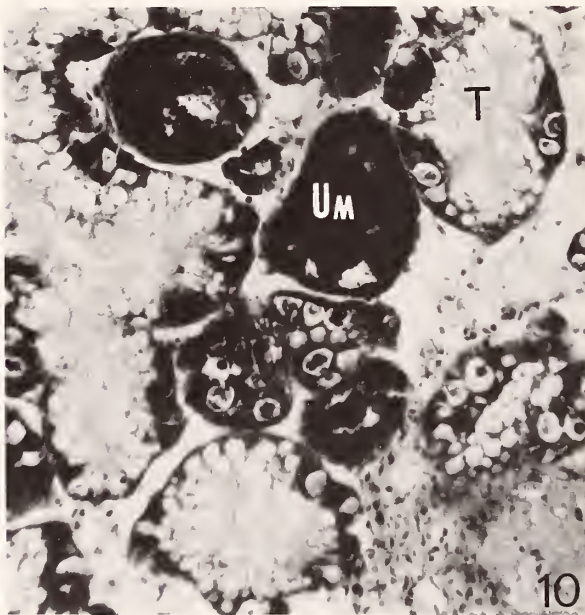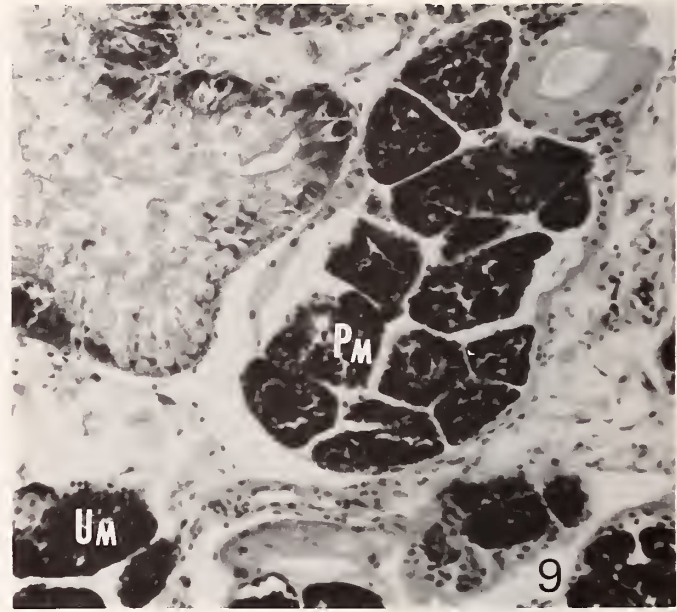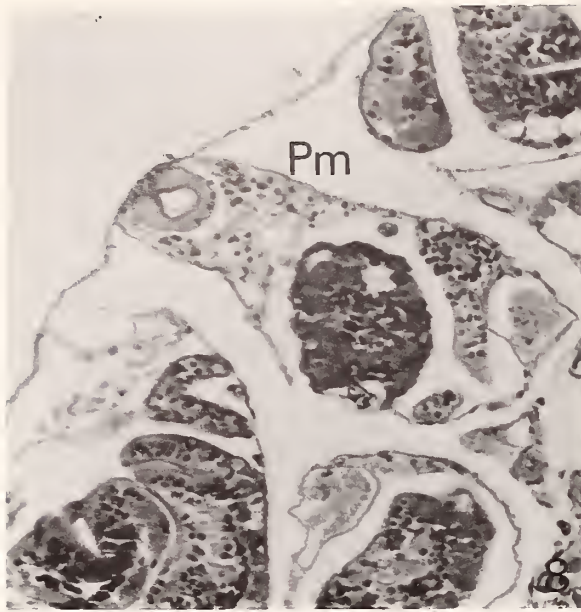
Explanation of Figures 8 to 11

Figure 8. *Philophthalmus megalurus* (Pm) infection in *Goniobasis virginica*. H&E, 100×.

Figure 9. *Philophthalmus megalurus* (Pm) redia and sporocyst of a ubiquita microphallid (Um). H&E, 450×.

Figure 10. Sporocysts of a ubiquita microphallid (Um) in the digestive gland (tubule, T) of *Goniobasis virginica*. H&E, 450×.

Figure 11. Double infection of the digestive gland with *Sphaeridiotrema globulus* (Sg) and a ubiquita microphallid (Um). H&E, 100×.
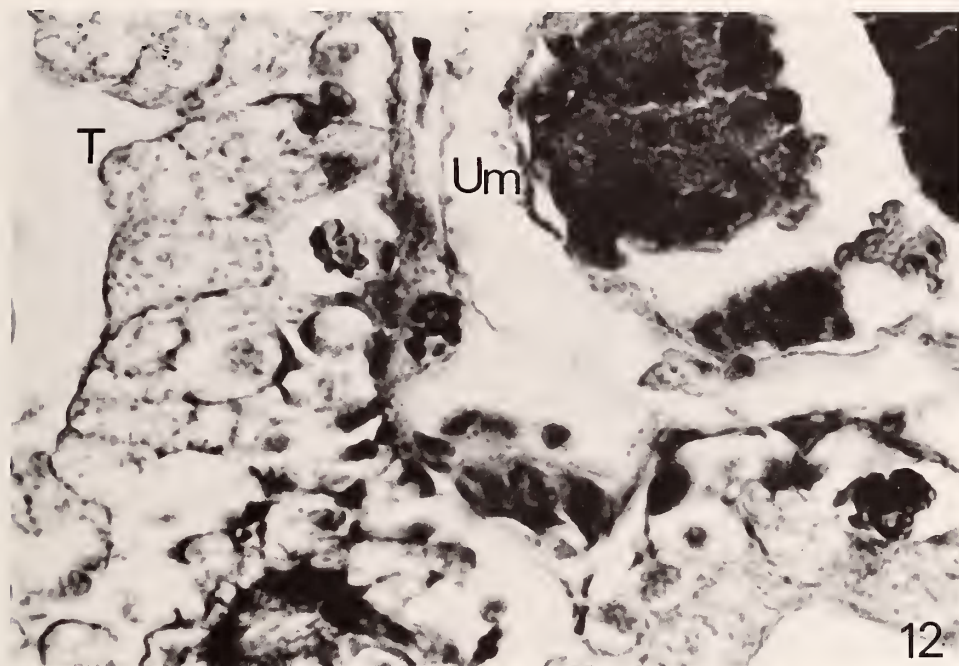
Figure 12

Sporocyst of a ubiquita microphallid (UM) abutted to a tubule (T) in the digestive gland of *Goniobasis virginica*. H&E, 1000×.

One difficulty in studies employing naturally infected snails is that the sequence of pathology cannot be determined nor the long term consequences of infection on the initial changes that occur at the beginning of infection.

The primary method of cell destruction and removal by rediae of *Sphaeridiotrema globulus* in the digestive gland appeared to be through ingestion. This was indicated by the presence of host cellular debris in the redial gut. CHENG (1963c) observed similar changes in *Helisoma trivolvis* infected with *Echinoparyphium* sp. rediae. The rediae and sporocysts reported in our study no doubt exerted mechanical pressure on the digestive gland tubules as evidenced by constricted tubular lumens. It is also possible that the excretory products of the rediae and sporocysts had a lytic effect on host tissue, as evidenced by the clear zone around living trematodes. The clear zones were devoid of cells and these zones may be edematous.

A hemocyte response to dead *Sphaeridiotrema globulus* rediae was noted but no reaction occurred in response to living larval trematodes. Many workers have noted little or no cellular response to living trematode larvae in their molluscan hosts (CHENG, 1963b; CHENG & BURTON, 1965; JAMES, 1965; FENG, 1967; LOKER, 1978). Mechanisms by which living parasites inhibit the ability of the mollusk to recognize them as foreign is unknown (FONT, 1980). Dead trematodes lose this capability and are attractive to the hemocytes of the snail. MEULEMAN (1972) noted infiltration of hemocytes to foci of cellular necrosis as the result of trematode activity.

Impairment of the digestive gland function may occur as the result of *Goniobasis virginica* heavily infected with larval trematodes. Whether or not this impairment has an effect on survival of the snail is not known. CHENG & SYNDER (1962a) reported that *Helisoma trivolvis* heavily infected with *Glypthelmins pennsylvaniensis* can survive the infection.

Many of the effects of intramolluscan parasites on the host's metabolism are known but difficult to generalize (BECKER, 1980). The digestive gland of uninfected *Goniobasis virginica* contains glycogen which appears as granular material scattered throughout the cytoplasm. In snails infected with either sporocysts or rediae there was a decrease in the glycogen content of the digestive gland cells. Concurrent with the depletion of host glycogen was an increase in the glycogen content of developing parasites. This depletion suggests the utilization of host glycogen by these larval digeneans. The rediae probably derive most of their glycogen by ingesting host cells. They may also absorb nutrients through their body walls, but glycogen is most likely too large to pass through the digenean body wall (CHENG & SYNDER, 1963). Glycogen from the host digestive gland is most likely hydrolyzed to monosaccharides which pass out of gland cells into intertubular spaces. A decrease in neutral lipid content was also evident in

snails infected with either sporocysts or rediae. This also suggests utilization of host lipid by these larval parasites. The effects of glycogen and lipid depletion have been extensively studied in various molluscan hosts by CHENG (1962, 1963a, b, c, 1965), CHENG & SYNDER (1962a, b, 1963), CHENG & BURTON (1966), JAMES (1965), PORTER et al. (1967), NEGRUS (1968), READER (1971a), and ROBSON & WILLIAMS (1971).

Keratin, a fibrous protein, was found to be substantially depleted only in snails infected with *Philophthalmus megalurus*. This protein was reported by DIXON (1965) in the metacercarial cyst wall of *Fasciola hepatica* which encysts on vegetation. *Philophthalmus megalurus* also encysts on vegetation and the keratin uptake by this parasite may be in preparation for the encystment process. The deposition of a keratin sheath around the autolytic redia of *Sphaeridiotrema globulus* appears to be an attempt by *Goniobasis virginica* to wall off the parasite.

ACKNOWLEDGMENTS

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